cont measured by its ability to correct the prolonged clotting time of plasma prepared from patients with hemophilia A. a_1

Please replace the ~~fourth~~ third paragraph starting at page 1 with the following:

The development of inhibitory antibodies (inhibitors) to fVIII is a serious complication in the management of patients with hemophilia A. Alloantibodies develop in approximately 25% of patients with hemophilia A in response to therapeutic infusions of fVIII. In previously untreated patients with hemophilia A who develop inhibitors, the inhibitor usually develops within one year of treatment, although it can occur at any time. Additionally, autoantibodies that inactivate fVIII can occur in non-hemophiliacs in a variety of clinical settings including the postpartum period, in systemic lupus erythematosus, in chronic lymphocytic leukemia, and in elderly females. This condition is called acquired hemophilia. a_2

Please replace the second paragraph starting at line 7, page 2 with the following:

fVIII inhibitors are measured clinically by the ability of the patient's plasma to inhibit fVIII in normal plasma. The standard test is the Bethesda assay. One Bethesda unit is defined as the dilution of patient plasma required to reduce the fVIII level by 50%. a_3

Please replace the third paragraph starting at line 11, page 2 with the following:

A molecule is said to be *antigenic* when it binds to antibodies and *immunogenic* when it can induce an immune response. The immunogenicity of a molecule depends on the B cell repertoire, T cell help and suppression, and the major histocompatibility complex, which together determine the concentration and binding affinity of antibodies for an antigenic site. If a fVIII molecule could be constructed that did not bind to the inhibitory antibodies in a patient's plasma, it would useful therapeutically. Additionally, if a fVIII molecule could be constructed that is less immunogenic than wild-type human fVIII, i.e., could significantly lower the 25% incidence of inhibitor development, it a_4

cnt
Q4

would be safer than wild-type human fVIII. This molecule would have general applicability in the hemophilia A population.

Please replace the fourth paragraph starting at line 21, page 2 with the following:

Q5

Inhibitory antibodies to fVIII bind to either the A2, A3, or C2 domains of fVIII and disrupt specific functions associated with these domains. The A2 epitope is located within a linear sequence bounded by residues Arg484-Ile508. The C2 epitope has been localized to a sequence bounded by residues Glu2181-Val2243. The A3 epitope has not yet been mapped. The fact that fVIII epitopes are limited in number and can be mapped to the amino acid sequence level makes it possible to design strategies to produce low antigenicity and low immunogenicity fVIII molecules. We have already reduced the antigenicity of fVIII by replacing epitopes with non-human fVIII sequences and by site-directed mutagenesis of amino acids within fVIII epitopes.

Please replace the first paragraph starting at line 1, page 3 with the following:

Q6

Viruses, such as the human immunodeficiency virus (HIV), elude the immune system by varying epitopes that are recognized by antibodies. HIV contains an exterior envelope glycoprotein, gp120, which is targeted by the immune system in its attempts to rid the body of virus. HIV reduces the immunogenicity of gp120 using a post-translational process in which a polysaccharide is linked to asparagine residues. This process is called N-linked glycosylation because N is the single letter code for the amino acid asparagine. When the immune system makes antibodies to the existing glycosylated epitope, HIV responds by mutation vary its N-linked glycosylation sites. This reduces the immunogenicity of the virus. Similarly, the immunogenicity of fVIII could be reduced by altering the epitope by glycosylation. Additionally, the structure recognized by existing antibodies would be altered, reducing the antigenicity of the molecule.